DISCUSSION

ROLAND CUMMINGS, M. D. (523 West Sixth Street, Los Angeles).—In every case of a suspected appendicitis, the clinician has had to consider the possibility of the symptoms resulting from ovarian disease, and many times he concludes that the ovary is at fault regardless of his inability to explain the pathology.

It is interesting, therefore, to read the description of the disease under consideration written almost seventy years ago by Doctor Priestly. His outline of the symptomology is very complete, and his observation of left-sided pain and tenderness much more common than has been observed by Doctor Hirshfeld. His theory of the ovarian changes during the monthly cycle were very shrewd when compared to the complete knowledge of the cycle existing today.

Rhodenburg and Hellman were able to produce cystic degeneration of the ovaries by injecting corpus luteum. Is it possible, then, that for some unknown reason there has been the production of an excessive amount of corpus luteum in the patients previous to the development of the cysts?

The point in the history regarding the time of the monthly cycle in which this trouble occurs emphasized by the author I wish to reëmphasize, as the diagnosis will often hinge upon the correctness of this information.

I feel grateful to Doctor Hirshfeld for his clear delineation of the mechanism of the production of the symptoms due to dysovulation.

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ROY E. THOMAS, M. D. (1136 West Sixth Street, Los Angeles). — To the internist the chief interest of Doctor Hirshfeld's paper lies in those points which aid in the differential diagnosis between the accident described and acute appendicitis.

In the male the diagnosis of acute appendicitis rarely offers much difficulty; but in the female, after puberty, such a diagnosis is often a real problem. Among the more common conditions to be ruled out are acute salpingitis, extra-uterine pregnancy, and ruptured Graafian follicle or corpus luteum cyst. If the diagnosis of rupture of a Graafian follicle can be established with reasonable certainty, the patient is spared an unnecessary laparotomy.

Of first importance in the differential diagnosis of ruptured Graafian follicle simulating appendicitis is an accurate history. One or more previous attacks, of short duration, the absence of previous dyspepsia, the onset of pain in the lower abdomen rather than the epigastrium, and occurring approximately fourteen days after the beginning of the previous menstrual period, are against appendicitis as a cause of the attack.

On examination, the patient with a ruptured follicle is apt to be in a disturbed emotional state, presenting evidence of the autonomic upset described by Doctor Hirshfeld. Palpation of the abdomen shows less muscle spasm than is usually found in appendicitis. Why the pain is usually referred to the right lower quadrant when the pathology may be in either ovary, I cannot say.

Inasmuch as serious bleeding rarely occurs except with the rupture of a corpus luteum cyst (which comprised less than 20 per cent of Doctor Hirshfeld's series), it would seem that whenever the diagnosis seems reasonably certain, these cases should be treated conservatively, unless definite signs of progressive hemorrhage are present.

Dysovulation should be considered as a possible factor in the etiology of many functional disturbances in the female, and certainly in all cases presenting acute lower abdominal pain as a prominent symptom. Doctor Hirshfeld's paper is a timely one.

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FRED S. MODERN, M. D. (1135 Pacific Mutual Building, Los Angeles). — Dysovulation is a syndrome which has been known under various names, such as "intermenstrual pain" and "Mittelschmerz," for some sixty-odd years; but Doctor Hirshfeld's paper is, to my knowledge, the first which adequately summarizes and discusses the condition. It must be a comparatively frequent condition, as the author collected twenty-two cases from his own private practice. It must also undoubtedly be frequently overlooked because its symptoms overlap with other more common abdominal disorders from which it is difficult to distinguish, the diagnosis depending on a careful history rather than on the physical findings during an attack.

Dysovulation is essentially a disorder of follicular rupture during ovulation. Symptoms may occur through failure of the follicle to discharge the ovum, and the increase of tension within the follicle due to an increased amount of follicular fluid. Hyperestremia and hyperluteinization may occur as a consequence with their ensuing train of autonomic symptoms due to the excess estrin production and delayed menstruation, or even amenorrhea may follow due to a persistent corpus luteum, if the follicles should fail to involute poly (micro) cystic ovaries may result. If, however, follicular rupture does occur, dependent on the stage of follicular development, we have the picture of an acute abdominal episode either due to a chemical peritonitis or to a more or less extensive intra-abdominal hemorrhage.

Therapeutically the problem is discouraging. Doctor Hirshfeld points out that he has attempted to treat four patients conservatively with opotherapy, all of whom relapsed. The acute attacks associated with true intraperitoneal complications have, as a matter of course, to be treated operatively, unfortunately without definite hope of an ultimate recovery unless mutilated by an oöphorectomy. My own personal experience extends only to a total of five cases of intermenstrual pain which could be tentatively interpreted as dysovulation. In three of the cases the condition was associated with a lowered basal rate and other symptoms indicating myxedema. These three cases responded brilliantly to the administration of thyroid extract and emmenin, the oral anterior pituitary-like preparation developed by Collipp. The other two cases had a normal basal rate and organotherapeutic treatment proved a flat failure.

The recent development of potent endocrine preparations has placed a powerful weapon at the disposal of the medical profession for good or bad. Experience is still lacking as to what side effects some of these preparations might have in long-continued use. Potent estrin-like extracts have been proved, at least in animal experimentation, to have a sclerosing effect on the ovary and its fibrous tunic. It stands to reason that dysovulation might be an undesired side effect, at times, of previous treatment with an estrin-like substance.

BRUCELLA ABORTUS AGGLUTININS*

A STUDY OF THEIR INCIDENCE IN THE BLOOD OF THE GENERAL POPULATION OF A CITY AND SEVERAL RURAL COMMUNITIES IN CALIFORNIA

By C. EMELIA PETERSON, M.A.
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DISCUSSION *by K. F. Meyer, Ph.D., San Francisco.*

THE following is a report of a comparative study of the frequency of occurrence of Brucella abortus agglutinins in the blood of human beings, collected from the general population of a large urban community and from three rural communities in California. The urban group consisted of patients who registered in the out-patient clinics, and the wards of Lane and Stanford hospitals in

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San Francisco. From these individuals 399 specimens of blood submitted for Widal tests, and 1,631 specimens submitted for Wassermann tests, were studied. The rural group were residents of Kern, Orange, and San Bernardino counties, because the records of the State Department of Health had shown the highest incidence of undulant fever to occur in these localities among the counties of California. Through the courtesy of the county health officials of these communities, 1,336 specimens of blood were submitted from the patients of the various county hospitals: 522 from Kern County, 441 from Orange County, and 373 from San Bernardino County.

HISTORICAL

Considerable work has been done by various investigators on the incidence of Brucella abortus agglutinins in human blood, with wide variations in results. Many have reported positive reactions in dilutions of 1-5 and 1-10, but the majority of investigators are agreed that such low titers are of doubtful clinical significance. McAlpine and Mickle [1] examined 10,157 samples of human blood sent to the Bureau of Laboratories of the Connecticut State Department of Health, and reported sixty-three (0.6 per cent) reacting in a titer of 1-100 or higher. In another series [2] of 10,102 serums collected for the Wassermann test, sixty-four (0.6 per cent) were positive in dilutions of at least 1-100. Of 4,050 samples of human serum submitted for the Wassermann test, Carpenter and Boak [3] found 7.3 per cent to contain abortus agglutinins, but only thirteen (0.32 per cent) agglutinated Brucella abortus in titers ranging from 1-90 to 1-1215. Spray and Hess [4] examined 2,521 serums sent to the State Hygienic Laboratory of West Virginia for routine Kahn and Widal tests, finding twenty-eight (1.1 per cent) to react in a titer of 1-100. Bayne-Jones [5] checked blood serums from 3,716 individuals and reported sixty-four (1.72 per cent) to contain Brucella abortus agglutinins in a dilution of 1-100 or higher. Kilduffe, Fitzpatrick, and Wilson [6] tested 500 samples received in New Jersey for Widal and Wassermann tests, finding only one (0.2 per cent) to give a positive reaction in a dilution of 1-160. Gilbert and Coleman,[7] in studying a large series of 81,848 specimens, reported that 369 (0.4 per cent) reacted with Brucella abortus in dilutions of 1-80 or higher. Of 1,186 blood serums received for Widal tests, seventy (5.9 per cent) were positive in a dilution of at least 1-80. In the series herein reported, a minimum titer of 1-80 was selected.

PROCEDURE

Antigens were prepared from bovine, caprine, and porcine types of Brucella abortus, all of which were obtained from Dr. K. F. Meyer of the George Williams Hooper Foundation for Medical Research. Agar cultures in Blake bottles were collected in sterile physiological salt solution containing 0.5 per cent phenol. After the bacteria had been killed, which was usually within a few days, the suspensions were standardized to a density of No. 4, barium sulphate nephelometer.[8] The initial macroscopic agglutination tests were set up in dilutions of 1-10, 1-20, 1-40, 1-80, and 1-160, using buffered salt solution adjusted to pH 7.0 for dilution, as described by Evans.[9] A saline control was run with each series, and each batch of fresh antigen was tested with normal serum before use. All agglutination tests were placed in an incubator at 37 degrees centigrade over night, and were read the next morning; and only those showing a definite clumping with a clear supernatent fluid were regarded as positive. Brucella abortus Burr, a bovine strain, was used for all initial tests. If there was sufficient serum, positive tests were repeated in dilutions ranging from 1-10 to 1-10,240, and with caprine and porcine strains of Brucella abortus. All sera which showed hemolysis were discarded.

RESULTS

Of 399 samples of blood sent to the bacteriology laboratory of Lane and Stanford hospitals for the Widal test over a period of approximately eight years, only three (0.75 per cent) agglutinated Brucella abortus. Two of these were from patients who lived outside of the city of San Francisco; one, from a child who was known to have drunk raw milk, agglutinated the porcine strain in a dilution of 1-640; the other, from a farmer engaged in raising cattle in the San Joaquin Valley, in the vicinity of Stockton, agglutinated the porcine strain in a titer of 1-20,480. No information is available concerning the third patient, whose serum agglutinated the bovine strain in a dilution of 1-640.

No positive agglutination tests for Brucella abortus were observed in 1,631 blood serums received from the Wassermann Laboratory of Lane and Stanford hospitals over a period of seven months.

Thus, of a total of 2,030 serums collected in our institution, three (0.14 per cent) agglutinated Brucella abortus in titers ranging from 1-640 to 1-20,480.

Of, 1,336 specimens of blood serum received from Kern, Orange, and San Bernardino counties over a period of three months, twelve (0.89 per cent) contained agglutinins for Brucella abortus. Of 373 specimens received from San Bernardino County, two (0.53 per cent) were positive; of 522 specimens received from Kern County, only two (0.38 per cent) agglutinated Brucella abortus; and of 441 specimens received from Orange County, eight (1.81 per cent) were positive. The titers of the positive sera varied from dilutions of 1-80 to 1-2,560.

Therefore, of a total of 3,366 human blood serums collected from urban and rural communities in California, fifteen (0.44 per cent) reacted with Brucella abortus in a dilution of at least 1-80.

Referring to Table 1, it will be found that of a total of twelve reactors, seven were males and five were females. Three fell within the 20 to 29 age group, three within the 30 to 39 age group, one in the 40 to 49 age group, and two in the 50-59 age group. The ages of three are unknown.

TABLE 1.—*Human blood serums received from rural communities which gave positive agglutination tests with Brucella abortus in titers of 1–80 or higher.*

Patient	Sex	Age	Occupation	Contact with Domestic Animals	Use of Raw Milk	Use of Pasteurized or Canned Milk	Agglutination Titers Brucella Abortus		
							Bovine	Caprine	Porcine
H. K.	M.	34	Farmer	Milked cows in Kansas prior to August 1931	Sometimes		1-320	1-160	1-160
C. H. S.	M.	24	Interne		1 pint daily		1-160	1-320	1-160
R. A. D.	F.	?	?	?	?	?	1-160		1-80
H. R.	F.	21	Housewife		On cereals only	Pasteurized milk—on cereals only	1-160	1-160	1-160
R. S.	M.	23	Laborer	?	?	?	1-640	1-160	1-160
W. H. B.	M.	?	Service station operator		Small amount daily		1-5120	1-640	1-2560
R. A. A.	M.	30	Oil worker		1 glass cream daily. Very little milk		1-80	1-40	1-80
J. B.	F.	?	Housewife		½ pint daily		1-2560	1-640	1-2560
A. K. H.	M.	30	Florist		Used daily but in no cerain amount	Pasteurized milk daily but in no certain amount	1-2560	1-640	1-640
H. J.	M.	53	Tractor driver	Patient sold dairy herd in Kansas because contagious abortion present	Five or six glasses daily		1-320	?*	?*
S. R.	F.	52	Housewife			One pint to one quart pasteurized milk daily	1-80	?*	?*
M. C.	F.	42	Packer			Canned milk	1-80	1-40	1-80

* There was insufficient serum to run tests with the caprine and porcine strains.

As to occupation, three were housewives and there was one each in the following occupation groups: hospital interne, florist, farmer, laborer, service-station operator, oil worker, tractor driver, and packer. The occupation of one was unknown. Two individuals had had contact with dairy cows, one of whom knew that his herd was infected with infectious abortion. Eight used raw milk or cream. Positive agglutination tests with the bovine strain of Brucella abortus checked fairly closely with the caprine and porcine varieties.

COMMENT

The percentage of positive results reported in this study is low as compared with the findings of many investigators, but checks favorably with the results of those who considered titers of at least 1-80 or 1-100 to be of diagnostic significance. In addition to the positive reactions already reported, there was a group of twenty-five serums that agglutinated Brucella abortus Burr in dilutions of 1-5 and 1-10, a few reacting in dilutions of 1-20 and occasionally in slightly higher dilution. These tests were all negative when repeated with the three types of abortus antigen and, had they been included, the percentage of reactors in our total number of 3,366 sera would have been raised from 0.44 to 1.18 per cent.

The findings of this investigation indicate that the incidence of Brucella infection is greater in rural districts than in the city. That no positive tests occurred in the Wassermann series collected in San Francisco is not surprising, as pasteurized milk is generally used in the Bay region, there being relatively little raw milk except certified milk on the market. It was an interesting observation that positive reactions in significant titers occurred in subjects from rural communities, including two of the Widal series in San Francisco. The highest titers occurred in individuals who used raw milk or cream, or who came in contact with domestic animals, whereas the low titers occurred generally in persons who ordinarily used pasteurized or canned milk.

CONCLUSIONS*

In this study, an agglutination titer of at least 1-80 was considered to be diagnostic of Brucella infection.

Of 2,030 human serums representative of a city population collected in various departments of the Stanford Medical School, three (0.14 per cent) agglutinated Brucella abortus in a titer of at least 1-80, two of which were known to be from individuals living outside of the city of San Francisco. Of 1,336 specimens received from rural communities, twelve (0.89 per cent) were positive for Brucella abortus. Of the collective total of 3,366 human serums from rural and urban communities, fifteen (0.44 per cent) reacted with Brucella abortus in a titer of at least 1-80 or higher.

These results show that there is a greater incidence of Brucella abortus agglutinins in human blood in rural than in urban communities in California.

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DISCUSSION

K. F. Meyer, Ph.D. (Hooper Foundation, University of California, San Francisco).—The careful study on the Brucella agglutinins by Miss C. E. Peterson furnishes ample evidence against the so-called nonspecific agglutination. The data presented leave no doubt that the presence of agglutinins against the Brucella varieties is chiefly met within areas in which the infection is endemic in the livestock, and human exposure by contact or ingestion is not infrequent. It is now universally recognized that the occurrence of agglutinins in the sera of human beings is, in reality, the result of infection, and not the expression of nonspecific agglutination. In order to secure some information relative to the frequency of human brucelliasis, a great many laboratories have conducted agglutination tests on the sera which had been submitted for the Widal test.

In accepting a titer of 1:100 and above as diagnostic, the following figures have been reported:

	Per Cent
Denmark (Kristensen, 1931)	9.8
Sweden (Olin, 1931)	0.5
Norway (Vogelsang)	2.0
Esthonia (Schlossmann, 1931)	4.5
Ireland (Bigger, 1932)	2.0
England (Wilson, 1932)	3.5
Switzerland (Grumbach, 1933)	6.1

On the other hand, the systematic examination of sera collected from afebrile persons detected not only the manifest, but also the latent infections. The figures, which were thus obtained, reflect to a certain extent the endemization of an occupational group, or certain population strata. Wilson (1932) has admirably reviewed the literature. He finds that in the United States approximately 2 per cent in 20,000 serum tests yielded agglutination reactions in dilutions of from 1:40 to 1:50, in Great Britain and Ireland 1.4 per cent in 6,600 tests. In Sweden, Olin found 6.1 per cent of 3,000 Wassermann sera positive. These serologic surveys have never taken into consideration the natural population grouping, since relatively few sera of children are submitted for the Wassermann test. Further, the persistence of the agglutinins, following a manifest or latent infection, are not known and for each case are doubtless of variable duration. Assuming that the agglutinins persist for approximately two years, the two per cent positive sera would justify the conservative estimate that approximately one per cent of the population suffers annually from clinical or latent brucelliasis.

Somewhat more instructive are the detailed serologic studies which have been made on occupational groups exposed to the risk of Brucella contact infections. Employees of slaughterhouses, of the dairy trade, and of stock farms, and groups of veterinarians have been tested. The percentage of positive agglutination reactions in 4,128 was on the average 15.6 per cent. However, it must be emphasized that neither the agglutination nor the complement fixation reactions furnish a true picture of the number of latent infections in an occupational group. In all probability the same holds true for the population as a whole. With the aid of the allergic skin and the phagocytic index test, the percentage of latent infections in the personnel connected with the meat-packing industry may be as high as 91 to 95 per cent.

The significance and the interpretation of the Brucella agglutination reaction is quite often a matter of considerable difficulty. It demands the closest coöperation between the clinician and the serologist. In doubtful cases it should be combined with the complement fixation and the phagocytic index test. The allergic skin test requires tested reagents and a great deal of experience.

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COMPARATIVE CARCINOGENIC POTENCY OF COMMON AGENTS

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THAT malignant tumors often develop at the site of repeated or continuous irritation of tissues has been observed by clinicians and investigators for a long time, both in animals and in man.[1] Not every form of chronic irritation, however, is capable of producing cancerous changes. Most workers who have used physical and chemical nonspecific irritants have failed to observe true malignant changes.[2]